Efficacy of SLICE® Premix (0.2% emamectin benzoate) for Reducing Infestations of Salmincola spp. on Freshwater-Reared Rainbow Trout

Journal:	*North American Journal of Aquaculture*
Manuscript ID:	UNAJ-2011-0115
Manuscript Type:	Article
Date Submitted by the Author:	01-Dec-2011
Complete List of Authors:	Bowker, James; US Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program Carty, Daniel; U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program Wandelear, Niccole; U. S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program Swee, Wesely; Missouri Department of Conservation, Maramec Spring Fish Hatchery Schaffer, Jim; SeaPac of Idaho, Magic Springs Trout Hatchery LaPatra, Scott; Clear Springs Food, Inc,
Keywords:	Rainbow trout, SLICE, emamectin benzoate, Salmincola californiensis, Control, Infestation

Efficacy of SLICE® Premix (0.2% emamectin benzoate) for Reducing Infestations of *Salmincola* spp. on Freshwater-Reared Rainbow Trout

James D. Bowker*, Daniel G. Carty, Niccole Wandelear, Jim Schaffer[1], Wesley Swee[2] and Scott E. LaPatra[3]

U.S. Fish and Wildlife Service

Aquatic Animal Drug Approval Partnership Program

4050 Bridger Canyon Road

Bozeman, Montana 59715

[1]SeaPac of Idaho

Magic Springs Trout Hatchery

3069 Magic Springs Road

Hagerman, Idaho 83332

[2]Maramec Spring Fish Hatchery

Missouri Department of Conservation

21304 Maramec Spring Drive

St. James, Missouri 65559

[3]Clear Springs Food, Inc.

1585-A Clear Lakes Grade

Buhl, Idaho 83316

*Corresponding author, jim_bowker@fws.gov

Running Title: Control of *Salmincola* on rainbow trout

Abstract

Salmincola spp. are ectoparasitic crustacean copepods of major concern in wild and cultured salmonids. These parasites can cause respiratory distress and facilitate the entry of secondary pathogens. Of particular concern in the United States is *S. californiensis*, which can infest all *Oncorhynchus* spp. and is restricted largely to fresh water. Bath treatments with formalin and hydrogen peroxide have traditionally been used to control *S. californiensis* infestations in cultured salmonids; however, these treatments can be difficult to apply, expensive and stressful to fish, and discharge of formalin in hatchery effluent may be an issue. A more effective and efficient treatment method needs to be developed. SLICE® (0.2% emamectin benzoate; EB) is an in-feed treatment that has been shown to be effective for the control of sea lice infestations in seawater-reared farmed salmon and trout. We postulated that EB may also be efficacious for the control of parasitic copepods such as *S. californiensis* on freshwater-reared salmonids. Four trials were conducted to evaluate the effectiveness of SLICE®-medicated feed administered at a target dosage of 50 µg EB/kg fish biomass/d for seven consecutive days to control infestations of *S. californiensis* on freshwater-reared rainbow trout. At the end of each trial (after either a 30 or 42-d posttreatment period) copepod prevalence in treated tanks was substantially reduced compared to pre-trial prevalence recorded in the reference populations. Additionally, a significant difference was detected in mean abundance between treated and control groups, with a 79 – 96% reduction in mean abundance among fish offered the EB-medicated feed. Based on results of these trials, it

47 was concluded that SLICE® was efficacious in reducing infestations of *S. californiensis*
48 on freshwater-reared rainbow trout.

49 **Introduction**

50 Copepods are the most prevalent of the parasitic crustaceans affecting a wide
51 range of marine and freshwater finfish worldwide (Lester and Hayward 2006).
52 *Salmincola* spp. are parasitic copepods of major concern in wild and cultured salmonids.
53 It has been reported that the incidence and spread of these parasites can be exacerbated
54 by degraded environmental conditions such as increased water temperature, high organic
55 loading, low dissolved oxygen, and overcrowding (Sutherland and Wittrock 1985). Such
56 parasitic copepods often open portals of entry for secondary, opportunistic pathogens,
57 potentially increasing morbidity and mortality of fish (Cusack and Cone 1986; Bandilla et
58 al. 2006). Fish heavily infested with *Salmincola* spp. will flash, jump, or rub along hard
59 surfaces as they try to rid themselves of the parasites. Fish may become darker in color,
60 produce excessive mucus, exhibit fatigue in flowing water, and go off feed (Conley
61 1994). As a result, reduced growth and fecundity of fish populations may occur (Gall et
62 al. 1972; Sutherland and Wittrock 1985; Duston and Cusack 2002).

63 A *Salmincola* sp. of particular concern to salmonid culturists in the U.S. is *S.*
64 *californiensis.* This species is native to Pacific coastal drainages, but has been reported
65 as far east as New Jersey and West Virginia (Hoffman 1999; Sutherland and Wittrock
66 1985). This copepod infests all *Oncorhynchus* spp., but is restricted largely to fresh
67 water. The life cycle of *S. californiensis* is characterized by three phases including the
68 free swimming stage, an infective copepodid stage, the attached juvenile stage (chalimus

I- IV), and the adult. Adults include a large female, which attaches permanently to the host fish via a unique holdfast called a bulla, and a dwarf male, which attaches to the female, dies after mating, and is not parasitic *per se* (Roberts et al. 2004). The macroscopic (2.5 – 8.0 mm total length) female is pale yellow, normally with two egg sacs dangling from the body, and is the most conspicuous of the adult *S. californiensis* (Conley 1994). The copepod attaches primarily to the gills of fish, thus causing extensive gill damage (Kabata and Cousens 1977; Sutherland and Wittrock 1985; Lester and Hayward 2006). At high levels of infestation, this species can also be found on the fish body surface and opercula and in oral cavities (Sutherland and Wittrock 1985).

Bath treatments of formalin and hydrogen peroxide have traditionally been used to control infestations of parasitic crustaceans, however, these treatments can be difficult to apply, expensive and stressful to fish (Stone et al. 2000a). Additionally, most chemicals used for treatment are not approved for legal use in the U.S. Some fish culturists have resorted to manual removal of individual *S. californiensis* from fish but have had only marginal success (Roberts et al. 2004). Clearly, a more effective and efficient method needs to be developed to treat large numbers of cultured fish infested with *S. californiensis*.

SLICE® (0.2% emamectin benzoate; EB; Merck Animal Health, Summit, New Jersey) is an in-feed treatment developed for the control of sea lice (e.g., *Lepeophtheirus salmonis* and *Caligus elongatus*) infestations in farmed salmon and trout. It has been extensively tested to evaluate environmental safety, efficacy, and tolerance in Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, and brown trout *S. trutta* in the marine environment (Armstrong et al. 2000; Roy et al. 2000; Stone et al. 1999, 2000a,

2000b, 2000c, and 2002). Currently, SLICE® is approved for the control of sea lice in salmonids in the United Kingdom, Europe, Norway, Chile, and Canada. Emamectin is an avermectin developed initially for food crop use and is derived synthetically from avermectins produced by fermentation of the soil bacterium *Streptomyces avermitilis*. When EB is fed to fish, it is absorbed from the gut and distributed to a variety of tissues. When sea lice feed on the host fish, EB is taken into the tissues of the parasite, binds to ion channels of nerve cells and disrupts transmission of nerve impulses, resulting in paralysis and death of the copepods (BCCAHS 2007). In addition, EB is metabolized and excreted slowly by fish, resulting in an extended period, up to 9 weeks, of protection from sea lice (Stone et al. 2000a). Similar effectiveness was observed in studies by Hakalahti et al. (2004) evaluating the efficacy of EB to control *Argulus coregoni* on rainbow trout and by Duston and Cusak (2002) evaluating the efficacy of EB to control *S. edwardsii* in brook trout *Salvelinus fontinalis*. We postulated that SLICE® would be similarly effective for the treatment of other freshwater copepods, such as *S. californiensis*. Four trials were conducted to evaluate the effectiveness of EB-medicated feed administered at a target dosage of 50 µg EB/kg fish biomass/d for seven consecutive days to control infestations of *S. californiensis* on freshwater-reared rainbow trout.

Methods

Test facilities, test fish, and test article.—Trials 1 – 3 were conducted in southern Idaho at commercial fish production facilities rearing all-female populations of rainbow trout for market, and trial 4 was conducted at a Missouri Department of Conservation fish hatchery rearing mixed-sex populations of rainbow trout for sport fishing enhancement (Table 1). In trials 1 and 2, SLICE®-medicated feed was purchased from Skretting

(Vancouver, British Columbia, Canada). In trials 3 and 4, we obtained SLICE® premix from Merck Animal Health Corp. and top-coated the premix onto feed supplied by and typically fed at each test facility. To verify EB doses administered, we collected samples of medicated and control feeds and had the samples analyzed for EB concentration at Eurofins/AvTech Laboratories, Inc. (Portage, Michigan).

Experimental hypothesis and design.—In each trial, the null hypothesis was that (1) mean abundance of live adult female *S. californiensis* on fish in the EB-treated group was equal ($P \geq 0.05$) to that in the nontreated (control) group or that (2) percent reduction in mean abundance of live adult female *S. californiensis* was <90%. We chose to count live adult females only because males are not parasitic, and adult males and many of the immature life stages of both sexes can be difficult to see without magnification. Two other criteria had to be met to ensure the validity of trial results: (1) at the beginning of each trial, mean abundance (number of parasites counted in a group of fish divided by total number of fish in the group) and prevalence (percentage of fish in a group with one or more parasites) of live adult female *S. californiensis* had to be ≥3/fish and ≥70%, respectively; and (2) at the end of the trial, the infestation level (mean abundance and prevalence) in the control tanks had to be ≥50% of that observed at the beginning of the trial.

Completely randomized designs (Petersen 1985) were used to allocate fish from naturally infested reference populations held in raceways to test tanks (20 fish/tank) and assign treatment conditions to tanks (3 – 5 tanks/treatment, depending on the trial; Table 1). Each trial comprised a 1 – 12 d pretreatment acclimation period, 7 d treatment period, and 30 d (trials 1 and 2) or 42 d (trials 3 and 4) posttreatment period (Table 1). Based on

the results of trials 1 and 2 and information from the literature (Stone et al. 2000b and 2000c, Treasurer et al. 2002, Gunn et al. 2011), longer posttreatment periods for trials 3 and 4 were used in an effort to quantify potentially greater levels of treatment efficacy as measured by percent reduction in mean abundance. In each trial, feed was administered by hand twice daily during the treatment period and once or twice daily during the pre- and posttreatment periods. Feed was administered at 0.5% initial mean fish body weight (BW)/d (trials 1 – 3) or 1.0% initial mean BW/d (trial 4) and not adjusted for growth or mortality. Masking procedures were used in all trials to ensure individuals feeding fish and collecting crucial data (e.g., *S. californiensis* counts) did not know which tanks were treated and which were controls.

Experimental procedures.—Before a trial started, 30 fish from the reference population were impartially collected and euthanized in 200 mg/L tricaine methanesulfonate solution (tricaine; Tricaine-S®, Western Chemical, Inc., Ferndale, Washington; Finquel®, Argent Chemical Laboratories, Redmond, Washington). The number of *S. californiensis* on each fish was counted and specimens were collected for confirmation as *S. californiensis* by qualified personnel at the U.S. Fish and Wildlife Service, La Crosse Fish Health Center (Onalaska, Wisconsin) according to methods described in Margolis and Kabata (1988). Twenty of these 30 fish were also randomly selected and evaluated for fish health. Fish health evaluations included gross observations of major external and internal organs and tissues. Additionally, a skin scraping was taken and examined microscopically for the presence of other ectoparasites and bacteria. In all but trial 4, routine bacteriology was performed for presumptive diagnosis of fish pathogens historically found at each site.

Trial 1(Magic Springs Trout Hatchery, Hagerman, Idaho)—The reference population comprised approximately 500 adult female rainbow trout (Table 1) held for 12 d in a section of a single raceway. The raceway had a rearing volume of 21,404 L, a water inflow of 2,718 L/min and a water turnover rate of 7.6 exchanges/h. The reference population had an estimated infestation prevalence of 93% and mean ± SD abundance of 5.5 ± 4.6 *S. californiensis*/fish. A total of 200 reference population fish were impartially captured and allocated among 10 rectangular (2.10 m long × 0.52 m wide) concrete tanks (431.5 L volume, 37.9 L/min inflow). The tanks were supplied with first-pass water from the same source that supplied the reference population raceway; *S. californiensis* carrier fish were present in the water supply. The posttreatment period lasted 30 d. During the pretreatment period, fish that died were replaced with fish from the reference population to ensure 20 fish/tank were available at the start of the treatment period, however, no mortalities were replaced within 24 h of initiating treatment.

Trial 2 (Clear Springs Foods (CSF) Research and Development Facility (RDF), Buhl, Idaho) —The reference population comprised approximately 9,016 adult female rainbow trout (Table 1) held in a single raceway at the CSF Briggs East Facility (Buhl, Idaho). The raceway had a rearing volume of 74,417 L, water inflow of 6,796 L/min and a water turnover rate of 5.5 exchanges/h. Test fish were impartially captured, sedated in a 40 mg/L benzocaine solution (Benzoak®, ACD Pharmaceuticals, Alesund, Norway) and visually examined for *S. californiensis.* All fish with one or more of the parasites were placed in a subsection of the raceway to create a secondary reference population of approximately 200 infested fish. These fish were transported (~2 mi) in plastic containers (oxygen supplementation provided) to the CSF RDF, where most were

immediately transferred to eight tanks or used for pretreatment *S. californiensis* counts, collection of copepods for definitive identification to species, or fish health evaluations. Test tanks were circular and made of fiberglass (1.04 m diameter; 0.53 m water depth; 432.6 L/tank; water inflow, 41.6 L/min/tank). The tanks were supplied with third-pass water that was (to the best of our knowledge) devoid of *S. californiensis* carrier fish The secondary reference population had an estimated infestation prevalence of 100% and mean abundance of 7.9 ± 6.3 *S. californiensis*/fish. The posttreatment period lasted 30 d.

Trial 3(CSF RDF).—The reference population comprised approximately 7,540 adult female rainbow trout (Table 1) held in a single raceway at the CSF Briggs East Facility. The raceway had a rearing volume of 74,417 L, water inflow of 6,796 L/min and a water turnover rate of 5.5 exchanges/h. Approximately 230 reference population fish were impartially captured and transported to the CSF RDF. Methods of transfer, test tanks used, and source water supplied were the same as in trial 2. The reference population had an estimated infestation prevalence of 100% and mean abundance of 7.3 ± 6.0 *S. californiensis*/fish. The posttreatment period lasted 42 d.

Trial 4 (Maramec Spring Fish Hatchery, St. James, Missouri)—The reference population comprised approximately 1,007 adult, mixed-sex rainbow trout (Table 1) held in a single raceway. The raceway had a rearing volume of 95,009 L, a water inflow of 9,462 L/min and a water turnover rate of 6.0 exchanges/h. A total of 120 test fish were impartially captured and allocated to six rectangular "holding boxes" (2.44 m long × 0.91 m wide × 0.91 m high) made of aluminum or wood with mesh screen bottoms and sides. Positioned in two rows of three in the raceway, these tanks were supplied with the same first-pass water supplied to the reference population raceway. Fish infested with *S.*

californiensis were present in this water supply. The reference population had an estimated infestation prevalence of 77% and mean abundance of 6.6 ± 12.6 *S. californiensis*/fish. The posttreatment period lasted 42 d.

Data collection.—Mortality, general fish and feeding (appetite) behaviors, water temperature, and dissolved oxygen concentration data were collected daily during the pretreatment and treatment periods but less frequently during the posttreatment periods. Hardness, alkalinity, and pH were measured at the beginning of a trial from a water sample collected from the reference population raceway and at the end of a trial from a water sample collected from one arbitrarily selected tank. Measured water quality parameters were considered sufficient to rear healthy rainbow trout (Table 1). Mortalities were removed from each tank and recent mortalities or moribund (approaching death) fish were examined externally and internally (unless postmortem changes precluded necropsy). Skin scrapings were examined by light microscopy for ectoparasites and bacteria and samples of kidney tissue were collected for bacteriology (except in Trial 4).

General fish behavior was assessed and characterized as normal or abnormal for each tank, and any abnormal behaviors (e.g., flashing, piping at the surface) were described. Appetite behavior was observed and recorded twice daily during the treatment period and once daily during the posttreatment period. Feeding behavior was assessed on a 5-point ordinal scale based on the relative amount of feed offered that was consumed and aggressiveness of fish when offered feed (0 = no feed was consumed and fish showed no interest in feeding; 1 = approximately 25% of feed was consumed and fish showed little interest in feeding; 2 = approximately 50% of feed was consumed and fish showed moderate interest in feeding; 3 = approximately 75% of feed was consumed and fish

showed moderate interest in feeding and fed below the water surface; and 4 = approximately 100% of feed was consumed and fish fed aggressively, with some breaking the water surface during feeding). The numbers of *S. californiensis* were counted on all live fish remaining in test tanks at the end of each trial. Fish were collected individually, lightly sedated in 200 mg/L tricaine, and live, attached, adult female copepods on gills, opercula, skin, fins, and mouth were counted.

Data analyses.—At the end of each trial, mean abundance of *S. californiensis* was compared between treatment groups with a mixed-model, nested analysis of variance (ANOVA; $P < 0.05$; SYSTAT 2007; Bowker et al., in press). In this analysis, tank was the experimental unit, fish was the observational unit, treatment was the fixed factor, and tank nested within treatment was the random factor. To compensate for some *S. californiensis* counts of zero, the count for each fish was increased by one and $\log_e$-transformed before analysis. The least squares means from the ANOVA were back-transformed ($e^{\text{treatment group mean}}$) to geometric means, which were used to calculate percent reduction in mean abundance (treated group compared with control group).

$$\text{Percent reduction in mean abundance} = 100 - \left[100 \times \frac{(\text{geometric mean}_{\text{treated}} - 1)}{(\text{geometric mean}_{\text{control}} - 1)}\right]$$

This equation was based on Abbott (1925) and adapted from Stone et al. (2000a, 2000b, 2000c, and 2002) for log-transformed data and estimates the relative magnitude of the treatment effect achieved, which is useful for making biological effect-size comparisons (Nakagawa and Cuthill 2007) of treatment efficacy across trials conducted with a variety of fishes under a variety of experimental designs, parasite infestation levels, and environmental conditions.

At the end of each trial, mean cumulative mortality was compared between treatment groups with a mixed-effects logistic model fitted in SAS Proc GLIMMIX (Wolfinger and O'Connell 1993; SAS 2008; Bowker et al. 2010). The random effect of tank was modeled with an *R*-side covariance structure, and the difference between groups was considered significant if $P < 0.05$.

Results

Trial 1

Mean abundance of *S. californiensis* in treated tanks (3.3 ± 5.4/fish) was significantly ($P < 0.001$) different from that in control tanks (9.5 ± 8.1/fish; Table 2). Fewer than two copepods were found on 67% of the 83 treated fish alive at the end of trial compared with 18% of the 90 control fish (Figures 1 and 2). Percent reduction in mean abundance was 79%. Infestation prevalence (93% in the reference population) decreased substantially in treated tanks (mean, 43%; range, 33 – 53%) but remained high in control tanks (mean, 91%; range, 85 – 100%; Table 2).

Mortality occurred in both treatment groups; however, mean cumulative mortality in treated tanks (16%; range, 5 – 25%) was not significantly ($P = 0.290$ different from that in control tanks (10%; range, 0 – 20%). Approximately 26% of all mortalities occurred during the treatment period. During the treatment period, fish in all tanks ate approximately 75% of feed offered. During the posttreatment period, fish ate approximately 100% of feed offered. Throughout the trial, general fish behavior was characterized as normal in all tanks.

During pretrial fish health evaluations, *Ambiphrya* sp. was detected on six fish, and *Trichodina* sp. was detected on one fish but both of these parasites were found at very low levels (≤1 organism/skin-scrape slide). Cultured kidney tissue samples exhibited insignificant or no bacterial growth. Two treated fish that died during the treatment period were evaluated for cause of death and bacteria presumptively identified as *Aeromonas salmonicida* were cultured from the kidney tissue of one of these fish but the fish did not show clinical signs of furunculosis disease. One control fish that died during the treatment period was necropsied and appeared normal internally but externally had fungus on the body surface. Necropsies of fish that died during the posttreatment period were inconclusive for cause of death.

The mean analytically verified EB dose administered to fish was 42 µg EB/kg fish/d (84% of target). No EB was detected in control feed.

Trial 2

Mean abundance of *S. californiensis* in treated tanks (1.9 ± 4.2/fish) was significantly different ($P = 0.001$) from that in control tanks (6.6 ± 6.7/fish; Table 2). Fewer than two copepods were found on 78% of the 77 treated fish at the end of the trial compared with only 12% of the 81 control fish remaining (Figures 1 and 2). Percent reduction in mean abundance was 83%. Infestation prevalence (100% in the reference population) had decreased substantially in the treated tanks (mean, 48%; range, 40 – 60%) but had remained high in control tanks (mean, 98%; range, 91 – 100%; Table 2).

Also, at the end of the trial, mean percent cumulative mortality in treated tanks (2.5%; range, 0.0 – 10.0% per tank) was not significantly different ($P = 0.644$) from that

in control tanks (1.3%; range, 0.0 – 5.0% per tank). All mortalities occurred during the posttreatment period. General fish behavior was characterized as normal in all tanks, and fish in all tanks consumed approximately 75% of feed offered daily.

Pretrial fish health evaluations detected very low levels (≤1 parasite /skin-scrape slide) of *Gyrodactylus* spp. on three fish. Only one of the fish that died during the trial was necropsied; no bacteria were detected in the kidney tissue or spleen tissue imprints and cultures made from this fish.

The mean analytically verified EB dose administered to fish was 44 µg EB/kg fish/d (88% of target). No EB was detected in control feed.

Trial 3

Mean abundance of *S. californiensis* in treated tanks (1.3 ± 5.7/fish) was significantly different ($P < 0.001$) from that in control tanks (12.2 ± 13.7/fish; Table 2). Fewer than two copepods were found on 90% of the 62 treated fish alive at the end of the trial compared with only 7% of the 68 control fish (Figures 1 and 2). Percent reduction in mean abundance was 96%. Infestation prevalence (100% in the reference population) had decreased substantially in treated tanks (mean, 15%; range, 5 – 33%) but had decreased only slightly in control tanks (mean, 93%; range, 82 – 100%; Table 2).

Mean percent cumulative mortality in treated tanks (mean, 24.7%; range, 10.0 – 40.0% per tank) was not significantly different ($P = 0.376$) from that in control tanks (mean, 15.0; range, 0.0 – 30.0% per tank). Approximately 6% of all mortalities occurred during the treatment period. General fish behavior was characterized as normal in all

316 tanks. Fish in all tanks ate approximately 75% of feed offered during the treatment
317 period and 100% of feed offered during the posttreatment period.

318 Reference population fish appeared normal during pretrial fish health evaluations,
319 and there was no evidence of secondary ectoparasites or bacteria. Two fish that died
320 during the treatment period and 30 fish that died during the posttreatment period were
321 examined and considered apparently normal, except for the culture of soluble brown
322 pigmentation from kidney tissue samples that was consistent with *A. salmonicida*
323 although no clinical signs of furunculosis were observed.

324 The mean analytically verified EB dose administered to fish was 49 µg EB/kg
325 fish/d (98% of target). No EB was detected in control feed.

326 **Trial 4**

327 Mean abundance of *S. californiensis* in treated tanks (1.3 ± 3.8/fish) was
328 significantly different ($P = 0.017$) from that in control tanks (12.5 ± 22.9/fish; Table 2).
329 Fewer than two of the copepods were found on 82% of the 51 treated fish remaining at
330 the end of the trial compared with only 38% of the 42 control fish (Figures 1 and 2).
331 Percent reduction in mean abundance was 90%. Infestation prevalence (77% in the
332 reference population) had decreased substantially in the treated tanks (mean, 26%; range,
333 18 – 32%) but had remained near the pretrial level in control tanks (mean, 74%; range, 56
334 – 92%; Table 2).

335 Also, at the end of the trial, mean cumulative mortality in treated tanks (1.7%;
336 range, 0.0 – 5.0% per tank) was not significantly different ($P = 0.895$) from that in

control tanks (2.4 %; range, 0.0 – 7.1% per tank). All mortalities occurred during the posttreatment period. At the end of the trial, live fish remaining in each tank were hand counted and some fish were unaccounted for and presumably escaped or were taken by predators such as great blue herons. To more accurately reflect relative mortality, the number of fish unaccounted for in a tank was subtracted from the number of fish in that tank at the start of the trial. General fish behavior was characterized as normal in all tanks. Overall, fish in treated and control tanks ate approximately 50% of feed offered during the treatment period and 75% of feed offered during the posttreatment period. On treatment days 2 – 4 heavy rain increased turbidity of water in the raceway holding the tanks. Feed consumption was at least 75% on the first day of the treatment period, decreased over the next 4 d to as low as 0 or 25%, and then increased to 50 or 75% during the last 2 d of the treatment period.

Pretrial fish health evaluations of reference population found that fish appeared normal; no bacteria or secondary ectoparasites were detected in the skin scrapes and internal organs appeared mostly normal (e.g., spleen of one fish was granular and was enlarged in another fish). Postmortem changes precluded performing necropsies on fish that died during the trial.

The mean analytically verified EB dose administered to fish was 44 µg EB/kg fish/d (88% of target). No EB was detected in control feed.

Discussion

The goal of the each trial was to evaluate the effectiveness of SLICE® administered in feed at 50 µg EB/kg of fish/d for seven consecutive days to reduce a

359 natural infestation of *S. californiensis* in test populations of adult, freshwater-reared
360 rainbow trout. In all four trials, treatment significantly reduced mean abundance of the
361 copepods and substantially reduced infestation prevalence. Percent reduction in mean
362 abundance was ≥90% in trials 3 and 4 (42 d posttreatment periods) but was only 79% and
363 83% in trials 1 and 2 (30 d posttreatment periods), respectively. Although efficacy was
364 not conclusively demonstrated in trials 1 and 2 because we did not meet both of our null
365 hypothesis criteria, results from all four trials indicate that the EB treatment regimen
366 administered can reduce natural infestations of *S. californiensis* in freshwater-reared
367 rainbow trout. In addition, results indicate that a greater level of reduction is likely to be
368 observed after 42 d compared with the 30 d posttreatment period.

369 It is possible that treatment efficacy could have been enhanced in trials 2 and 3
370 because test fish were drawn from a production facility that was *S. californiensis*-positive
371 and transferred for testing to a research facility considered *S. californiensis*-negative. By
372 the end of trial 2, mean abundance of *S. californiensis* in the control group had decreased
373 16% compared with the pretreatment mean abundance level in the reference population.
374 In contrast, by the end of trial 3, mean abundance of *S. californiensis* in the control group
375 had increased 67% compared with the pretreatment mean abundance level in the
376 reference population. These results suggest that moving infested fish to a facility or
377 raceway that is free of *S. californiensis* will not substantially reduce an infestation in
378 those fish and is unlikely to have influenced apparent EB treatment efficacy in these
379 trials.

380 It is also possible that treatment efficacy could have been adversely affected in all
381 four trials because fish in treated tanks did not appear to eat full rations of feed during the

treatment period. Dustin and Cosack (2002) commented they were surprised that treatment with EB-medicated feed was effective in reducing an infestation of *S. edwardsii* in adult, freshwater-reared brook trout *Salvelinus fontinalus* because the fish had a poor appetite, indicating a relatively low actual dose that can have therapeutic value. They also stated the variability between fish relative to treatment efficacy was likely a result of the competitive feeding nature in the population, resulting in individual fish not consuming equal amounts of medicated feed. There are inherent difficulties in treating a population of sick fish with medicated feed to ensure that the therapeutic dose was properly administered due to the competitive nature of feeding a population rather than individual animals (Storey 2005; Samuelsen 2006). Failure of some of the less-aggressive fish to consume a sufficient amount of medicated feed might have been the reason that there was ≥ 15 copepods on 2 – 5 fish at the end of each trial. We speculate that if all fish had consumed a full ration of feed, or at least enough feed to deliver a therapeutic dose, that the relative number of fish in each trial with no copepods would have approached 100%.

The efficacy of EB to reduce infestations of other copepods on freshwater salmonids has been demonstrated by others. As previously stated, Duston and Cusack (2002) treated adult, freshwater-reared brook trout with SLICE®-medicated feed at the same dosage as that used in our study (50 µg EB/kg fish/d for 7 d) to control a natural infestation of *S. edwardsii*. In two independent experiments (7 d or 31 d posttreatment periods), efficacy was evaluated by comparing percent change in abundance between treated and control groups. In both experiments, mean abundance was significantly reduced (40 – 60%; $P < 0.05$) within the treated groups while increasing by

approximately 20% within the control groups. Hakalahti et al. (2004) administered SLICE®-medicated feed at the same dosage used in our study to captive-reared rainbow trout to control a natural infestation of *A. coregoni* and found that treatment significantly reduced abundance. Results from their study were comparable to results observed by Hanson et al. (2011) in which no *Argulus* spp. were found on koi carp (*Cyprinus carpio*) or goldfish (*Carassius auratus*) at the end of the 7-d treatment period using the same dosage of SLICE®-medicated feed. Results from our trials were more comparable to results from Armstrong et al. (2000) and Stone et al. (2000a, 2000c) in which SLICE®-medicated feed administered to replicate groups of Atlantic salmon at the same dosage was used to control natural infestations of sea lice (*L. salmonis* and/or *C. elongates*). At the end of each study, mean abundance in the treated group was significantly less than that in the control group. Additionally, Stone et al. (2000a) reported treatment efficacy (abundance of *L. salmonis* on Atlantic salmon in treatment tanks versus controls) of ≥90% for two successive weeks starting at 42 d posttreatment and Stone et al. (2000b) found successively higher percent efficacy relative to controls from day 1 through day 28 posttreatment.

At the end of two of our trials (1 and 3), mortality was relatively high in both treated and control tanks. Although differences between treated and control tanks were not significant, mortality was higher in treated tanks than in control tanks. Due to the similar cumulative mortality in treated and control tanks, as well as information from the literature, it was presumed that the elevated mortality was not due to EB and was likely due, in part, to the copepod infestation. Roy et al. (2000) observed no mortality in Atlantic salmon and rainbow trout fed diets containing a seven-fold higher EB dose than

administered in our trials. Although kidney tissue cultured from some dead/moribund treated and control fish collected in our trials produced bacterial colonies morphologically consistent with *A. salmonicida,* no clinical signs of furunculosis were noted in any test fish. According to CSF personnel and other commercial trout producers in the area (Tom Van Tassel, Magic Springs Trout Hatchery, ID, personal communication), chronically elevated mortality is frequently observed in rainbow trout production populations during severe *Salmincola* spp. infestations. In addition to the physical and respiratory stressors associated with *Salmincola* spp.-infestation (Kabata and Cousins 1977; Sutherland and Wittrock 1985), this and other ectoparasites can reduce disease resistance in fish and be vectors for secondary fish pathogens (Bowers et al. 2000; Piasecki et al. 2004; Bandilla et al. 2006).

In conclusion, the results of our four trials indicate that SLICE®, when administered in feed at a dosage of 50 µg EB/kg fish/d for 7 d, can significantly reduce natural infestations of *S. californiensis* in freshwater-reared rainbow trout. Additional trials conducted with posttreatment periods longer than 42 d may help identify the point in time at which maximum treatment efficacy is achieved.

Acknowledgments

We thank Tom Van Tassel, Magic Springs Trout Hatchery, Bill Shewmaker and Robin Burkhart of Clear Springs Foods, Inc., Research Division, and Missouri Department of Conservation's Ben Havens, Danielle Mocker, Jeff Koppleman, Jen Girondo, Josh Ward, and Ron Burke for help in conducting this study. Doug Ramsey, Rangen Feeds, Inc. (Buhl, Idaho), performed fish health evaluations during the study at

Magic Springs Trout Hatchery. Eric Leis, FWS La Crosse Fish Health Center, La Crosse, WI, identified *S. californiensis*. Merck Animal Health paid for analysis of EB in feed samples.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265-267.

Anderson, R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Armstrong, R., D. MacPhee, T. Katz, and R. Endris. 2000. A field efficacy evaluation of emamectin benzoate for the control of sea lice in Atlantic salmon. Canadian Veterinary Journal 41:607-612.

Bandilla, M., E. T. Valtonen, L. R. Suomalainen, P. J. Aphalo, and T. Hakalahti. 2006. A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. International Journal for Parasitology 36:987-991.

Bowers, J. M., A. Mustafa, D. J. Speare, G. A. Conboy, M. Brimacombe, D. E. Sims, and J. F. Burka. 2000. The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. Journal of Fish Disease 23:165-172.

BCCAHS (British Columbia Center for Aquatic Health Resources). 2007. SLICE®: action / use / effects. BCCAHS, Campbell River, British Columbia, Canada.

471 Bowker, J. D., D. Carty, and M. M. Dotson. In press. Efficacy of 35% PEROX-AID®
472 (hydrogen peroxide) to reduce an infestation of *Gyrodactylus salmonis* in
473 freshwater-reared rainbow trout. North American Journal of Aquaculture.

474 Bowker, J. D., and D. A. Erdahl. 2010. The efficacy of SLICE® premix (0.2%
475 emamectin benzoate) administered in feed to control *Salmincola* spp. on all
476 freshwater-reared salmonids. Study Protocol Number SLICE-09-EFF-SAL. U.S.
477 Fish and Wildlife Service, Bozeman, Montana.

478 Bowker, J. D., V. E. Ostland, D. Carty, and M. P. Bowman. 2010. Effectiveness of
479 Aquaflor (50% florfenicol) to control mortality associated with *Streptococcus*
480 *iniae* in freshwater-reared subadult sunshine bass. Journal of Aquatic Animal
481 Health 22:254-265.

482 Conley, D. 1994. Gill maggot disease (genus *Salmincola*). In AFS-FHS (American
483 Fisheries Society-Fish Health Section). FHS Blue Book: Suggested procedures
484 for the detection and identification of certain finfish and shellfish pathogens, 2007
485 edition. AFS-FHS, Bethesda, Maryland.

486 Cusack, R. and D. K. Cone. 1986. A review of parasites as vectors of viral and bacterial
487 diseases of fish. Journal of Fish Diseases 9:169-171.

488 Duston, J., and R. R. Cusack. 2002. Emamectin benzoate: an effective in-feed treatment
489 against the gill parasite *Salmincola edwardsii* on brook trout. Aquaculture 207:1-
490 9.

Gall, G. A. E., E. L. McClendon, and W. E. Schafer. 1972. Evidence on the influence of the copepod (*Salmincola californiensis*) on the reproductive performance of a domesticated strain of rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 101:345-346.

Gunn, C., D. G. Carty, P. G. Walker, P. A. Colburn, and J. D. Bowker. 20xx. Pilot Field Trial to Evaluate SLICE® (0.2% Emamectin Benzoate)-Medicated Feed to Reduce a Natural Infestation of *Salmincola californiensis* in Freshwater-Reared Rainbow Trout. North American Journal of Aquaculture

Hakalahti, A., Y. Lankinen, and E. T. Valtonen. 2004. Efficacy of emamectin benzoate in the control of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 69:197-204.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, second edition. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

Kabata, Z., and B. Cousens. 1977. Host-parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda; Lernaeopodidae). Journal of the Fisheries Research Board of Canada 34:191-202.

Latremouille, D. N. 2003. Fin erosion in aquaculture and natural environments. Reviews in Fisheries Science 11:315-335.

Lester, R. J. G., and C. J. Hayward. 2006. Phylum Arthropoda. Pages 466-565 *in* P. T. K Woo, editor. Fish diseases and disorders, volume 1: protozoan and metazoan infections, 2nd edition. CAB International, Wallingford, Oxfordshire, England.

Margolis, L., and Z. Kabata. 1988. Guide to the parasites of fishes of Canada. Part II—Crustacea: Canadian special publication of fisheries and aquatic sciences, vol. 101. Canada Department of Fisheries and Oceans, Ottawa.

Nakagawa, S., and I. C. Cuthill. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological Reviews 82:591-605.

Petersen, R. G. 1985. Design and analysis of experiments. Marcel Decker, Inc., New York.

Piasecki, W., A. E. Goodwin, J. C. Eiras, and B. F. Nowak. 2004. Importance of Copepoda in freshwater aquaculture. Zoological Studies 43:193-205.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U.S. Department of the Interior, U.S. Fish and Wildlife Service, Washington, D.C.

Post, G. W. 1987. Textbook of fish health, revised and expanded edition. T. F. H. Publications, Inc., Neptune City, New Jersey.

Roberts, R. J., K. A. Johnson, and M. T. Casten. 2004. Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. Journal of Fish Diseaes 27:73-79.

Roy, W. J., I. H. Sutherland, H. D. M. Roger, and K. J. Varma. 2000. Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss*

(Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184:19-29.

Samuelsen, O. B. 2006. Pharmacokinetics of quinolones in fish: a review. Aquaculture 255:55-75.

SAS. 2008. SAS Version 9.2. SAS Institute, Inc., Cary, North Carolina.

Stone, J., I. H. Sutherland, C. Sommerville, R. H. Richards, and K. J. Varma. 1999. The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Kroyer), infestations in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 22:261-270.

Stone, J., I. H. Sutherland, C. Sommerville, R. H. Richards, and R. G. Endris. 2000a. The duration of efficacy following oral treatment with emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer) in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 23:185-192.

Stone, J., I. H. Sutherland, C. Sommerville, R. H. Richards, and K. J. Varma. 2000b. Commercial trials using emamectin benzoate to control sea lice *Lepeophtheirus salmonis* infestations in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 41:141-149.

Stone, J., I. H. Sutherland, C. Sommerville, R. H. Richards, and K. J. Varma. 2000c. Field trials to evaluate the efficacy of emamectin benzoate in the control of sealice, *Lepeophtheirus salmonis* (Kroyer) and *Caligus elongatus* Nordmann, infestations in Atlantic salmon *Salmo salar* L. Aquaculture 186:205-219.

553 Stone, J., W. J. Roy, I. H. Sutherland, H. W. Ferguson, C. Sommerville, R. H. Richard,
554 and R. G. Endris. 2002. Safety and efficacy of emamectin benzoate administered
555 in feed to Atlantic salmon, *Salmo salar* L., smolts in freshwater, as a preventative
556 treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer).
557 Aquaculture 210:21-34.

558 Storey, S. 2005. Challenges with the development and approval of pharmaceuticals for
559 fish. The AAPS Journal 7:E335-E343.

560 Sutherland, D. R., and D. D. Wittrock. 1985. The effects of *Salmincola californiensis*
561 (Copepoda: Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo*
562 *gairdneri*. Canadian Journal of Zoology 63:2893-2901.

563 SYSTAT. 2007. SYSTAT 12.02. SYSTAT Software, Inc., Chicago, Illinois.

564 Treasurer, J. W., C. Wallace, and G. Dear. 2002. Control of sea lice on farmed Atlantic
565 salmon S. salar L. with the oral treatment Emamectin benzoate (SLICE). Bulletin
566 of the European Association of Fish Pathologists 22: 375-380.

567 USDHHS (U.S. Department of Health and Human Services). 2001. Guidance for
568 industry document No. 85: good clinical practices. U.S. Department of Health
569 and Human Services, Food and Drug Administration, Center for Veterinary
570 Medicine, Washington, D.C.

571 Wolfinger, R., and M. O'Connell. 1993. Generalized linear mixed models: a
572 pseudolikelihood approach. Journal of Statistical Computation and Simulation
573 48:233-243.

TABLE 1. Trial information, test fish, experimental design, mean analytically verified emamectin benzoate (EB) dose administered (µg/kg fish/d) and percentage of target dose, and water quality in four trials to evaluate the efficacy of SLICE®-medicated feed to reduce natural infestations of *Salmincola californiensis* on rainbow trout.

		Fish sex and size[a, b]		Experimental design and EB dose administered			
Trial number and site	Trial dates	Sex	Mean (SD) total length (mm)	Mean (SD) weight (g)	Number of tanks	Pre- and posttreatment durations (d)[c] Pre	Post	Mean EB dose administered (% of target dose)[d, e]
1 Magic Springs Trout Hatchery	Jun – Jul 2010	F	316 (22)	400 (89)	5 treated 5 control	12	30	42 (84%)
2 Clear Springs Research and Development Facility	Jun – Jul 2010	F	362 (32)	645 (184)	4 treated 4 control	5	30	44 (88%)
3 Clear Springs Research and Development Facility	Oct – Dec 2010	F	339 (38)	517 (178)	4 treated 4 control	5	42	49 (98%)
4 Maramec Spring Fish Hatchery	May – Jul 2011	M, F	484 (55)	1,474 (501)	3 treated 3 control	1	42	44 (88%)

(continued on next page)

Water quality[e]				
Mean (SD) water temperature (°C)	Mean (SD) dissolved oxygen (mg/L)	Mean hardness (mg/L as $CaCO_3$)	Mean alkalinity (mg/L as $CaCO_3$)	Mean pH
15.3 (0.2)	8.6 (0.3)	236	125	8.1
14.4 (0.2)	7.1 (0.4)	202	144	7.8
13.4 (0.4)	6.3 (0.4)	233	154	7.8
14.1 (0.4)	8.7 (0.5)	160	158	7.3

[a]F = female; M = male

[b]For each trial, fish size was determined from a sample of 30 fish collected from a reference population.

[c]Pre = pretreatment period; Post = posttreatment period

[d]Target EB dose was 50 µg/kg fish/d.

[e]Standard deviations for mean EB doses administered and mean water hardness, alkalinity, and pH measurements not provided because sample sizes were small (n = 3 medicated feed samples/trial, and n = 2 water samples/trial for hardness, alkalinity, and pH).

Table 2. Prevalence and mean abundance of *Salmincola californiensis* on fish in the reference population before the start of each trial and in treated and control tanks at the end of each trial, and percent reduction in mean abundance in treated tanks compared with control tanks at the end of each trial. Mean abundance values with different lowercase letter in each row are significantly ($P < 0.05$) different from each other; prevalence data were not subjected to formal statistical analysis.

	Mean (range) prevalence (%)			Mean (SD) abundance (number/fish)			Percent reduction in mean abundance
Trial	Reference population	Treated tanks	Control tanks	Reference population	Treated tanks	Control tanks	
1	93	43 (33-53)	91 (85-100)	5.5 (4.4)	3.3y (5.4)	9.5z (8.1)	79
2	100	48 (40-60)	98 (91-100)	7.9 (6.3)	1.9y (4.2)	6.6z (6.7)	83
3	100	15 (5-33)	93 (82-100)	7.3 (6.0)	1.3y (5.7)	12.2z (13.7)	96
4	77	26 (18-32)	74 (56-92)	6.6 (12.6)	1.3y (3.8)	12.5z (22.9)	90

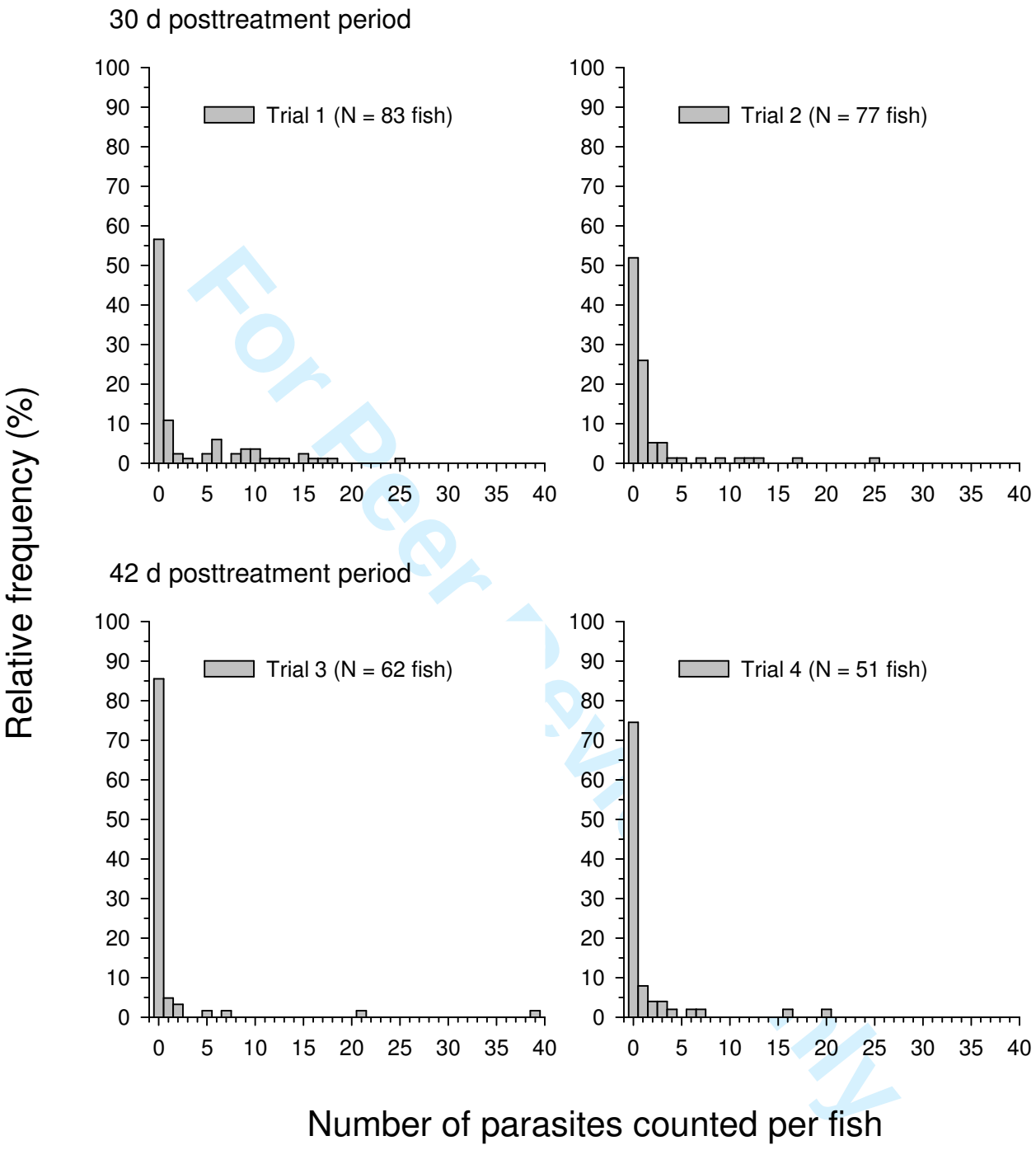

Figure 1. Relative frequency of live adult female *Salmincola californiensis* counts on treated fish at the conclusions of four trials to evaluate the efficacy of SLICE-medicated feed to reduce infestations of the parasite on freshwater-reared rainbow trout.

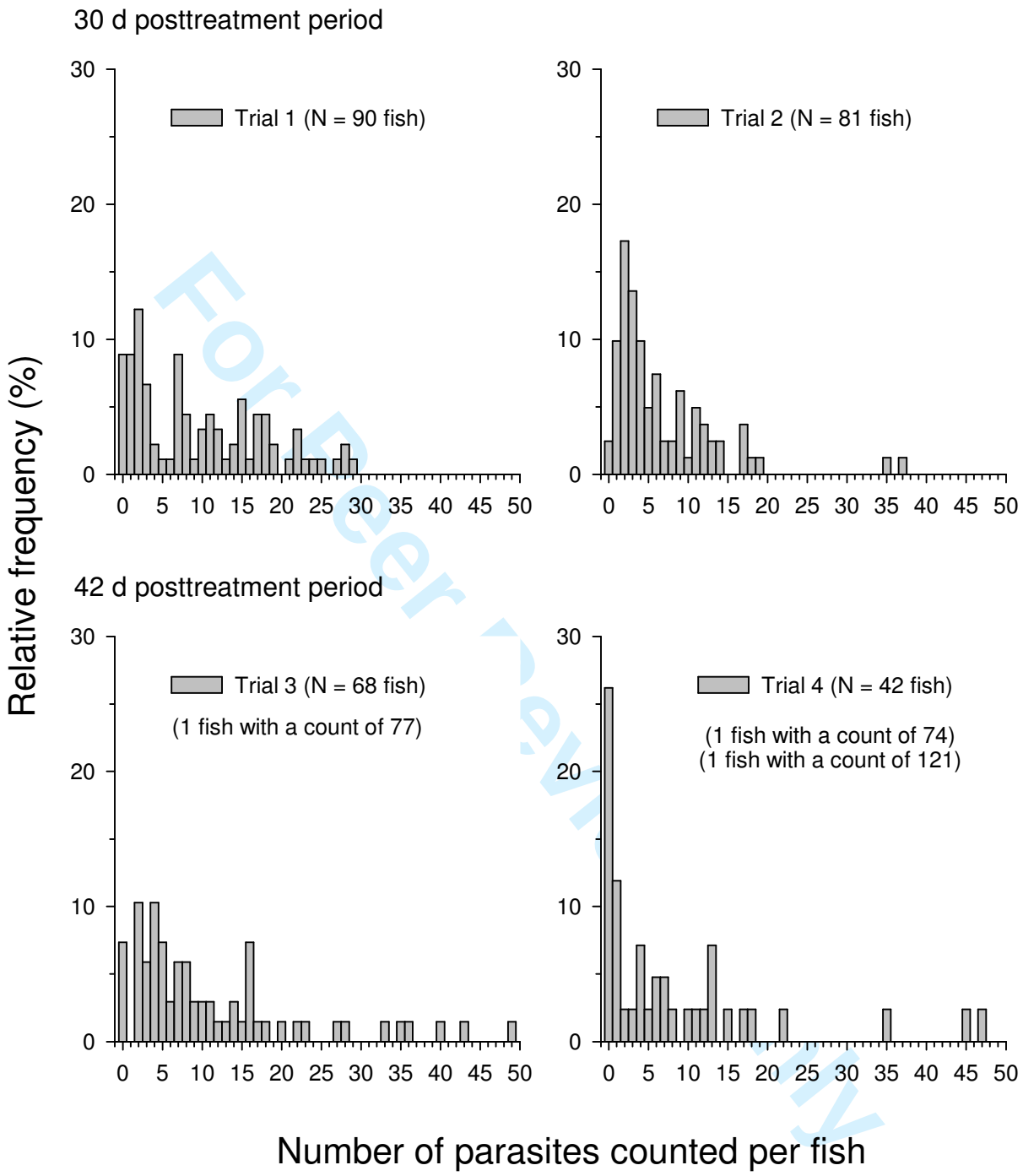

Figure 2. Relative frequency of live adult female *Salmincola californiensis* counts on control fish at the conclusions of four trials to evaluate the efficacy of SLICE-medicated feed to reduce infestations of the parasite on freshwater-reared rainbow trout.